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Discovery of lipoprotein lipase pI isoforms and contributions to their characterization

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ABSTRACT

Lipoprotein lipase (LPL) plays a pivotal role in lipid metabolism and is implicated in several pathophysiological conditions. A large number of LPL studies have been performed in rat, although the amount of information derived from direct study of the protein in this species is limited. Here we attempted to examine possible modifications of LPL using proteomic tools. By combining high-resolution two-dimensional gel electrophoresis and Western blot with biological mass spectrometry we demonstrate the coexistence of multiple LPL pI isoforms in rat heart. We studied the origin of this pI heterogeneity by: (1) comparison with the 2D pattern of LPL from post-heparin rat plasma (as a source of mature LPL); (2) protein dephosphorylation; (3) protein deglycosylation; and (4) partial sequencing of LPL isoforms. The results reveal that LPL pI heterogeneity does not correspond to different stages of intracellular maturation or protein phosphorylation. It can be partially explained by glycosylation, although other post-translational modifications must also be involved. We also report the first partial sequence to be obtained from direct study of rat LPL protein. These findings should be the basis for further research aimed at identifying the molecular differences between LPL isoforms and exploring their potential functional implications.

1. Introduction

Lipoprotein lipase (LPL) is a glycoprotein enzyme that plays a central role in lipid metabolism. Its primary function is the hydrolysis of circulating triacylglycerides (TAG), thus generating free fatty acids that are taken up by tissues. LPL is synthesized by parenchymal cells but it is functional in dimeric form bound through electrostatic interactions to heparan sulfate proteoglycans on the endothelial surface [1]. After a short half-life, LPL is released to the bloodstream and is finally taken up and degraded by the liver [2]. Abnormalities in LPL function have been associated with a number of pathophysiological conditions, including atherosclerosis, obesity, chylomicronaemia, Alzheimer's disease, and diabetes [3].

LPL is expressed in a variety of tissues and regulated in a tissue-dependent manner at transcriptional, translational and/or post-translational level [4]. It is believed that circulating TAG are mainly hydrolyzed by muscle and adipose tissue LPL.

Abbreviations: 2DE, two-dimensional electrophoresis; AP, alkaline phosphatase; CapLC-MS/MS, capillary liquid chromatography tandem mass spectrometry; IEF, isoelectric focusing; IPG, immobilized pH gradient; LPL, lipoprotein lipase; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; pI, isoelectric point; PTM, post-translational modification; TAG, triacylglyceride.

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However, heart is the tissue with the highest LPL expression [5] and heart LPL has been reported to play an important role in modulation of plasma TAG levels [6]. In support of this, heart specific LPL knock-out mice showed increased circulating TAG levels [7], whereas mice expressing LPL only in heart exhibited normal levels of TAG [6].

The rat is probably the most widely used animal model in LPL studies. However, most of the information on rat LPL available in protein databases is derived from nucleotide sequences and interpreted on the basis of homologies with LPL from other species [8] or other members of the triacylglyceride lipase family that have been characterized in greater detail, such as pancreatic lipase [9]. In contrast, little information has been derived from direct study of rat LPL protein.

We have studied LPL for several years in the rat, mainly at the physiological level [2,10,11]. Here we attempted to examine possible modifications of LPL using proteomic tools. Since LPL is a low-abundance protein, especially in terms of tissue proteome, the heart was chosen as a source for rat LPL and heparin-Sepharose affinity chromatography was used for partial enzyme purification. The use of high resolution two-dimensional gel electrophoresis (2DE) coupled with biological mass spectrometry (MS) allowed us to demonstrate now, for the first time, LPL pI isoforms and to present different approaches to study the origin of this pI heterogeneity.

2. Materials and methods

2.1. *Sample*

Male Wistar rats (Harlan Interfauna Ibèrica, Barcelona, Spain) were used. Hearts (28 g) were homogenized in 10 mM HEPES, 1 mM EDTA, 1 mM DTT, 0.3% w/v sodium deoxycholate, pH 7.5. In a different set of animals, post-heparin plasma (55 mL) was obtained after an injection of 50 U/kg heparin (USB Corporation, Cleveland, OH, USA). Commercial LPL from bovine milk was purchased from Sigma (St Louis, MO, USA). Procedures involving animals were approved by the Committee on Animal Bioethics and Care of the University of Barcelona and the Generalitat (Autonomous Regional Government) of Catalonia, Spain.

2.2. *Heparin-Sepharose chromatography*

LPL was partially purified using heparin-Sepharose affinity chromatography essentially as described elsewhere [12]. Briefly, chromatography was performed at 4 °C and at a constant flow rate of 0.25 mL/min. A heparin-Sepharose CL-6B column (0.7 cm×30 cm) was equilibrated with 10 mM Tris–HCl pH 7.4 buffer containing 30% v/v glycerol and 0.15 M NaCl. Approximately 55 mL of heart homogenate (0.8 g of total protein) or post-heparin plasma (3.9 g of total protein) was loaded into the column. Prior to application, the sample was adjusted to 0.15 M NaCl. In the case of post-heparin plasma sample sodium deoxycholate was also added to a final concentration of 0.1% w/v as reported [13]. After sample application, the column was washed and LPL was eluted by stepwise increase in NaCl concentration. Albumin was added to a small aliquot of each fraction (1 mg/mL final concentration) to preserve LPL activity [12]. Protein concentration [14] and LPL activity [15] were determined in fractions following standard procedures. LPL-containing fractions eluted with 1.5 M NaCl (100–150 μg of total protein) were pooled and frozen at −80 °C. For further experiments, proteins were precipitated using trichloroacetic acid followed by acetone washing as described elsewhere [16] and redissolved in an appropriate buffer.

2.3. *One-dimensional electrophoresis (1DE)*

Samples were solubilized in buffer (62.5 mM Tris–HCl pH 6.8, 10% v/v glycerol, 2% w/v SDS, 150 mM DTT and bromophenol blue), boiled for 10 min and applied to a 9% w/v polyacrylamide gel.

2.4. *Two-dimensional electrophoresis (2DE)*

After protein precipitation, partially purified LPL was solubilized in rehydration buffer (7 M urea, 2 M thiourea, 2% w/v CHAPS, 0.5% v/v IPG Buffer pH 3–10 or pH 6–11, and a trace amount of bromophenol blue) containing 18 mM DTT, sonicated as recommended [17], and frozen at −80 °C. Preparation of commercial bovine LPL for 2DE is described elsewhere [18]. The 7 cm immobilized pH gradient (IPG) strips, pH 3–10 or 6–11 (GE Healthcare, Uppsala, Sweden), were passively rehydrated overnight in 125 μL (350 μL for 18 cm IPG strips) rehydration buffer containing 1.2% v/v DeStreak Reagent (GE Healthcare, Uppsala, Sweden). Sample was applied to the rehydrated IPG strip by cup-loading at the cathode. The strips were focused according to the following protocol: linear ramp to 1000 V over 2 h, linear ramp to 5000 V over 1 h, and 5000 V for 25 kVh. When isoelectric focusing (IEF) was performed in 18 cm IPG strips pH 6–11 the last step was extended until 60 kVh. The focused IPG strips were incubated for 15 min in equilibration buffer (50 mM Tris, 6 M urea, 30% v/v glycerol, 2% w/v SDS and bromophenol blue) containing 65 mM DTT followed by a further 15-min incubation in the same equilibration buffer containing 135 mM iodoacetamide. This second equilibration step was not performed in strips used for later Western blot analysis, to avoid possible alterations in epitope and antibody recognition due to alkylation. The equilibrated strips were loaded in a 9% w/v polyacrylamide gel and sealed using a solution containing 0.5% w/v agarose, 25 mM Tris, 0.1% w/v SDS, 192 mM glycine and bromophenol blue.

2.5. *Silver staining*

Proteins were silver-stained using a procedure compatible with MS. Gels were fixed in 40% (v/v) ethanol and 10% (v/v) acetic acid for 30 min, sensitized in 30% (v/v) ethanol, 0.2% (w/v) sodium thiosulfate and 6.8% (w/v) sodium acetate for 30 min and washed with water (3×5 min). Then, gels were immersed in 0.25% (w/v) silver nitrate for 20 min, washed with water (2×1 min) and developed in sodium carbonate 2.5% (w/v) and 0.0148% (w/v) formaldehyde. Reaction was stopped with 1.46% (w/v) EDTA-$Na_2 \cdot 2H_2O$ for 10 min.

2.6. *LPL Western blot analysis*

After electrophoresis, proteins were transferred (1 h 100 V) to a nitrocellulose membrane and LPL was immunodetected by